THE INFLUENCE OF DIET ON THE TOXICITY AND MECHANISM OF ACTION OF ALPHA NAPHTHYLTHIOUREA

BY

RICHARD UGLOW BYERRUM

A.B., Wabash College, 1942

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
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INTRODUCTION

Alpha naphthylthiourea (ANTU) and certain other derivatives of thiourea have recently become of interest owing to the increasing use of these compounds as drugs and poisons.

Alpha naphthylthiourea is now being extensively employed as a rodenticide for the control of Norway rats. The compound is especially suitable as a rodenticidal agent because it is highly toxic to rats, somewhat less toxic to dogs, and much less toxic to other species.

In addition to its practical use, the administration of ANTU produces to a marked degree certain biological reactions which are common to other drugs and poisons. The most striking characteristics of ANTU poisoning are the development of pulmonary edema and massive pleural effusion, the ability of rats to tolerate many times the lethal dose of the rodenticide if sublethal quantities are first administered, and a marked species difference in the dose required to kill.

Although certain phases of the mechanism of action of ANTU and other thiourea compounds have been extensively investigated, the possibility that diet might be a factor in the species differences in susceptibility to ANTU, in the ability of animals to develop tolerance to the rodenticide, and in the ability of rats to detoxify ANTU, has not been adequately examined.

The present study was undertaken with the purpose of determining (a) the effect of variation of certain components of the diet upon the resistance of rats to ANTU, (b) the

effect of ANTU included in various diets upon growth rate, and upon the rate and extent of tolerance development, and (c) certain combinations of ANTU with enzymes and other biological compounds, the *in vivo* quantity of which might be affected by diet.

EXPERIMENTAL

Adult albino rats (Sprague-Dawley strain) were fed purified diets for varying time periods. At the end of any given feeding period the protection provided against ANTU was measured by intraperitoneal injection of the rodenticide dissolved in propylene glycol. Young male rats averaging 50 g. in weight were fed purified diets containing ANTU in experiments designed to measure growth rate and the development of tolerance.

RESULTS

The effect of variations in protein, carbohydrate, and fat, the three principal components of the diet, upon the resistance of rats to ANTU was studied. When diets in which the protein content was varied from 6% to 40%, the carbohydrate from 35% to 78%, and fat from 12% to 31% were fed for thirty days only the lowering of protein to 6% (a level inadequate for normal growth) appeared to have any effect upon the resistance of rats to ANTU. The dose of ANTU required to kill 50% of a group of animals eating this low protein diet (LD-50), was 3.8 mg. per kg. which was found to be significantly less than the control LD-50 of 6.8 mg. per kg. The decrease in resistance noted after feeding the 6% protein diet, however, was not great and does not appear to be a major factor in the species difference in lethal dose which might be caused by diet.

DuBois, Holm, and Doyle (1,2) found that the injection of the amino acid cysteine simultaneous with or up to 4 hours after ANTU administration protected rats against an LD-100 dose (8 mg. per kg.). In the present study it was found

that increasing the cystine content of the diet to 1% provided rats with an immediate protection against ANTU which disappeared after ten days of feeding. The LD-50 was 12.2 mg. per kg. after feeding this diet for 5 days, and 11.8 mg. per kg. after 10 days. When the 1% cystine diet was fed for longer periods, however, the initial resistance was lost, and after 30 days, the LD-50 was 6.0 mg. per kg. Increasing the cystine content of the diet to 5% gave no more protection against ANTU poisoning than had been furnished by 1% cystine in 10 days. It was also noted that a 5% level of cystine offered protection against ANTU only so long as sufficient additional choline chloride was included in the diet to give normal growth.

When the copper content of the diet was increased to 7 mg. per 100 g. rats received no detectable protection against ANTU poisoning after a 30 day feeding period. The LD-50 of ANTU for rats receiving copper was 6 mg. per kg. which is identical with that for the controls.

Iodine added either to the diet or to drinking water gave rats enormous protection against ANTU poisoning. When potassium iodide was fed at a level of 5.34 mg. of iodine per g. of diet for ten days, an LD-50 of 93 mg. per kg. was obtained following the injection of ANTU. The protection provided by iodine in this case guarded against some 15 times the median lethal dose for control animals. It was found that the amount of protection resulting from iodine was a function of the amount of iodine ingested by the animals prior to the time of injection of ANTU. The amount of protection afforded by a given amount of iodine was dependent upon the length of the feeding period. Thus, the ingestion of a given amount of iodine during a short feeding period afforded greater protection than the same quantity of iodine given in lower concentrations over an extended feeding period. This effect is probably accounted for by the rapid excretion of iodine.

By measurement of the growth rate of young rats it was found that ANTU was a growth inhibitor. The growth rate of the animals was inversely proportional to the quantity of ANTU incorporated in the diet within the dosage limits employed (0.02%-0.08%). It was found that addition of cystine to diets containing ANTU increased the growth rate of rats.

These studies indicate that rats are able to develop enough tolerance to certain levels of ANTU to show a steady gain in weight after about 10 days. When ANTU was increased gradually by addition of 20 mg. of ANTU every 5 days to each 100 g. of diet, rats were able to tolerate doses as large as 165 mg. per kg. per day without serious loss of weight.

It has been indicated by Toennies (3) that thiourea compounds might react with sulfhydryls to form disulfides. In an effort to determine some biochemical changes related to ANTU poisoning a measurement was made of oxidizable sulfhydryl compounds in liver and lung tissue of three normal species and in the tissues of rats injected with the rodenticide. It was found that the frog, a species very resistant to ANTU, had the greatest tissue content of both lung and liver sulfhydryl compound. The rat, a sensitive species, had a relatively low level of liver sulfhydryls. It was demonstrated that acute poisoning by ANTU definitely lowered the oxidizable tissue sulfhydryl content of rat tissue.

It has been shown that cystine provides rats with some protection against ANTU poisoning. A study of some biochemical changes resulting from feeding large amounts of cystine was undertaken. The oxidizable sulfhydryl compounds, total cystine, and succinic dehydrogenase activity of rat liver were measured in animals receiving various diets containing cystine. It was found that high dietary cystine, in addtion to causing fatty degeneration of the liver, resulted in an increase in total cystine and oxidizable sulfhydryls. These changes were accompanied by a reduction in liver

succinic dehydrgenase activity. The addition of choline chloride to the diets containing cystine completely prevented the increase in oxidizable sulfhydryls, reduced the increase in total cystine, and to some extent prevented the reduction in succinic dehydrogenase activity of rat liver.

SUMMARY

1. It has been shown that a 6% protein diet lowered the resistance of rats to ANTU poisoning while cystine and, to a much greater extent, iodine increased the resistance of rats to ANTU.

2. The effect of ANTU fed in the diet upon growth rate has been determined, and it was found that ANTU is a growth inhibitor. It was noted that rats may develop tolerance to two different levels of ANTU fed in the diet in about 10 days while animals fed a diet in which the ANTU concentration was periodically increased developed tolerance to as much as 165 mg. per kg.

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APPENDIX I

THE INABILITY OF TRANS CROTONIC ACID TO REPLACE THREONINE IN THE DIET FOR GROWTH OF YOUNG RATS

INTRODUCTION

Rose and associates (1) have indicated that 10 amino acids are essential in the diet of young rats for adequate growth. Omission of any of these, with the exception of arginine, results in a decline in weight followed eventually by death. It is possible, however, to substitute a closely related derivative for one amino acid in a diet in which mixtures of pure amino acids are fed to young rats. It has been shown by Cox and Rose (2) that β-4-imidazoleacrylic acid, the α,β unsaturated analogue of histidine, cannot replace that amino acid in the diet. Similarly it was shown by Lambert and Rose (3) that isopropylacrylic acid cannot be substituted for leucine in the diet of young rats.

The present study was undertaken with the purpose of determining the effect upon growth of substituting crotonic acid, the α,β unsaturated analogue of threonine, for threonine in the diet of young rats.

EXPERIMENTAL

Albino rats weighing between 45 and 60 g. were fed a diet in which all of the nitrogen was supplied as a mixture of purified amino acids. One group of rats, the control, was given a diet containing adequate quantities of all the essential amino acids for growth. A second group was provided a diet in which threonine was replaced by an equimolar quantity of trans crotonic acid. The third group of animals, the negative control, received neither threonine or trans crotonic acid. As a result of growth rate studies in which the weight of the

young animals eating these diets was observed every 4 days for a period of 28 days, it was noted that trans crotonic acid cannot replace threonine in the diet for growth of young rats. Those animals receiving trans crotonic acid lost weight at a rate equal to the negative controls.

SUMMARY

Experimental data have shown that trans crotonic acid is unable to replace threonine in the diet of the rat for purposes of growth.

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VITA

The author was born September 22, 1920, in Aurora, Illinois, and received his elementary and high school education in Warrenville and Wheaton, Illinois. He entered Wabash College in 1938 and received the Bachelor of Arts Degree in June of 1942. He enrolled in the Graduate School of the University of Illinois in September of 1942 as a Teaching Assistant in Chemistry and held this appointment until April of 1944 when he was employed at the University of Chicago Toxicity Laboratory as a Research Assistant in Pharmacology under the National Defense Research Committee. He continued work on this wartime project, which in February of 1945 was placed under the direction of the Chemical Warfare Service. He was enrolled *in absentia* in the Graduate School of the University of Illinois in October, 1945.